

CLAIM AMENDMENTS

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of claims:

1. (currently amended) A method for generating a population of variant DNA molecules in bacterial cells, said method comprising:
 - (a) transferring a donor vector into a bacterial cell capable of homologous recombination, wherein
 - (i) said donor vector comprises a donor recombination module comprising, in the following order from 5' to 3': a first donor DNA sequence and a second donor DNA sequence, and
 - (ii) said bacterial cell comprises a target vector comprising a target recombination module comprising, in the following order from 5' to 3': a first target DNA sequence; a negatively selectable marker; and a second target DNA sequence, wherein said first donor DNA sequence is homologous to said first target DNA sequence, and said second donor DNA sequence is homologous to said second target DNA sequence, ~~and~~
wherein at least one of (1) the first donor DNA sequence and the first target DNA sequence are not identical to each other, or (2) the second donor DNA sequence and the second target DNA sequence, are not identical to each other; and
 - (b) selecting for a population of bacterial cells within which recombination between the donor vector and the target vector has occurred, such that the cells do not contain the negatively selectable marker,thereby generating a population of variant DNA molecules in bacterial cells.
2. (original) The method of claim 1, wherein the donor vector further comprises a conjugative transfer sequence.
3. (previously presented) The method of claim 2, wherein the donor vector is transferred by conjugative transfer.

4. (previously presented) The method of claim 1, wherein the donor vector is transformed into the bacterial cell.

5. (original) The method of claim 3 or 4, wherein the donor vector is a suicide vector.

6. (original) The method of claim 1, wherein the target vector is integrated into the bacterial cell genome.

7. (previously presented) The method of claim 1, wherein the donor vector is transferred into the bacterial cell via a phage particle.

8. (previously presented) The method of claim 1, wherein the negatively selectable marker comprises a conditionally lethal sequence, and selecting for a population of bacterial cells in step (b) comprises selecting against said conditionally lethal sequence.

9. (previously presented) The method of claim 1, wherein: i) the target vector further comprises a reporter gene sequence downstream of the second target DNA sequence; ii) the negatively selectable marker is a polar insert sequence which prevents expression of the downstream reporter gene, such that the loss of said polar insert results in expression of the reporter gene; and iii) the step of selecting for a population of bacterial cells which do not contain the negatively selectable marker comprises selecting for expression of said reporter gene.

10. (original) The method of claim 1, wherein the negatively selectable marker in the target recombination module comprises a unique restriction endonuclease recognition site.

11. (previously presented) The method of claim 1, wherein selecting for the a population of bacterial cells which do not contain the selectable marker comprises amplifying DNA of the cells to determine whether the negatively selectable marker is absent from the cells.

12. (original) The method of claim 1, in which the donor vector further comprises a positively selectable marker.

13. (currently amended) A method for generating a population of variant DNA molecules in bacterial cells, said method comprising:

(a) transferring a donor vector into a bacterial cell which is capable of homologous recombination, wherein:

(i) said donor vector comprises a donor recombination module comprising, in the following order from 5' to 3': a first non-functional fragment of a selectable-marker; a first donor DNA sequence; and a second donor DNA sequence;

(ii) said bacterial cell comprises a target vector comprising a target recombination module comprising, in the following order from 5' to 3': a second non-functional fragment of a selectable-marker; a first target DNA sequence; and a second target DNA sequence,

wherein said first donor DNA sequence is homologous to said first target DNA sequence, and said second donor DNA sequence is homologous to said second target DNA sequence, and recombination between said first non-functional fragment of a selectable-marker and said second non-functional fragment of a selectable-marker results in a functional selectable marker, ~~and~~

wherein at least one of (1) the first donor DNA sequence and the first target DNA sequence are not identical to each other, or (2) the second donor DNA sequence and the second target DNA sequence, are not identical to each other; and

(b) selecting for a population of bacterial cells within which recombination between the donor vector and the target vector has occurred, such that the cells contain the functional selectable marker,

thereby generating a population of a variant DNA molecules in bacterial cells.

14. (previously presented) The method of claim 13, wherein the donor vector is transferred by conjugative transfer.

15. (previously presented) The method of claim 13, wherein the donor vector is transformed into the bacterial cell.

16. (original) The method of claim 14 or 15, wherein the donor vector is a non-replicating plasmid.

17. (original) The method of claim 13, wherein the target vector is integrated into the bacterial cell genome.

18. (currently amended) The method of claim 13, wherein the donor vector is transferred into the bacterial cell via a phage particle.

19. (original) The method of claim 13, in which the donor vector further comprises a positively selectable marker.

20. (previously presented) The method of claim 19, further comprising prior to step (b):

(c) selecting for a population of bacterial cells comprising the positively selectable marker of the donor vector.

21. (previously presented) The method of claim 1, further comprising:

(c) selecting said population of bacterial cells of step (b) for a desired phenotype.

22. (previously presented) A method for optimizing a phenotype comprising the method of claim 21, further comprising:

(d) repeating steps (a) - (c),

wherein the target recombination module used in step (d) is obtained from a bacterial cell selected in step (c).

23. (original) The method of claim 1 or 13, in which the donor vector further comprises a third donor sequence, located 3' to the first donor sequence and 5' to the second donor DNA sequence.

24. (original) The method of claim 23, wherein the third donor sequence comprises a negatively selectable marker.

25. (previously presented) The method of claim 22, in which the target recombination module of step (d) is identical to the target recombination module of step (a).

26. (previously presented) The method of claim 22, in which the target recombination module of step (d) is different from the target recombination module of step (a).

27. (previously presented) The method of claim 1, 13, or 22, further comprising, prior to step (a), the step of mutagenizing the donor vector.

28. (previously presented) The method of claim 21, further comprising, prior to step (a), the step of mutagenizing the donor vector.

29. (original) The method of claim 27, wherein the step of mutagenizing the donor vector is carried out in vitro.

30. (original) The method of claim 28, wherein the step of mutagenizing the donor vector is carried out in vitro.

31. (previously presented) The method of claim 27, wherein the step of mutagenizing the donor vector is carried out in vivo.

32. (original) The method of claim 28, wherein the step of mutagenizing the donor vector is carried out in vivo.

33. (original) The method of claim 1, 13, or 22, wherein the donor vector is a suicide vector.

34. (original) The method of claim 21, wherein the donor vector is a suicide vector.

35. (original) The method of claim 1, 13, or 22, wherein the bacterial cell is an E. coli cell.

36. (original) The method of claim 21, wherein the bacterial cell is an E. coli cell.

37-44. (canceled)

45. (previously presented) The method of claim 13, further comprising:

(c) selecting said population of bacterial cells of step (b) for a desired phenotype.

46. (previously presented) A method for optimizing a phenotype comprising the method of claim 45, further comprising:

(d) repeating steps (a) - (c),
wherein the target recombination module used in step (d) is obtained from a bacterial cell selected in step (c).

47. (previously presented) A method of claim 46, in which the target recombination module of step (d) is identical to the target recombination module of step (a).

48. (previously presented) The method of claim 46, in which the target recombination module of step (d) is different from the target recombination module of step (a).

49. (previously presented) The method of claim 46, further comprising, prior to step (a), the step of mutagenizing the donor vector.

50. (previously presented) The method of claim 45, further comprising, prior to step (a), the step of mutagenizing the donor vector.

51. (previously presented) The method of claim 49, wherein the step of mutagenizing the donor vector is carried out in vitro.

52. (previously presented) The method of claim 50, wherein the step of mutagenizing the donor vector is carried out in vitro.

53. (previously presented) The method of claim 49, wherein the step of mutagenizing the donor vector is carried out in vivo.

54. (previously presented) The method of claim 50, wherein the step of mutagenizing the donor vector is carried out in vivo.

55. (previously presented) The method of claim 46, wherein the donor vector is a suicide vector.

56. (previously presented) The method of claim 45, wherein the donor vector is a suicide vector.

57. (previously presented) The method of claim 46, wherein the bacterial cell is an *E. coli* cell.

58. (previously presented) The method of claim 45, wherein the bacterial cell is an *E. coli* cell.

59. (previously presented) The method of claim 12, further comprising prior to step (b):

(c) selecting for a population of bacterial cells comprising the positively selectable marker of the donor vector.